Scientific and clinical frontiers of gastroenterology and hepatology

This forward looking conference took place at the Royal College of Physicians on 23 November 1992. Credit for arranging the programme and selecting a panel of inspiring speakers goes to Humphrey Hodgson, Michael Farthing and Carol Seymour.

The programme was divided into the following sessions: 1. Transport processes: mechanisms and implications; 2. Genetic disorders; 3. Cancer surveillance: high risk groups; 4. Destructive hepatic processes.

Transport processes

Stomach

Dr Andrew Garner (ICI Pharmaceuticals, Macclesfield) surveyed the pharmacology of the parietal cell. The parietal cell is unique: not only can it generate the highest ion gradient (10^6) encountered in the body, it must also cope with the consequences of such activity, namely, exposure of the luminal surface to 0.15M HCl, and disposal of the equivalent amount of base from the cytoplasmic compartment. Acid secretion by the parietal cell is controlled by a complex sequence of neurohumoral stimuli: histamine, released by the paracrine cells, acts on the H2 (histamine 2) receptor to generate intracellular cAMP; ACh (acetylcholine), released by nerve endings, acts on M3 (muscarinic 3) receptors to increase intracellular calcium; and gastrin, which acts on CCK_B (cholecystokinin B) receptors, also increases intracellular calcium. Experimental studies of the tubulovesicular membrane of the parietal cell, which contains the H^+,K^+-ATPase (proton pump), have led to the development of the model of parietal cell function depicted in Fig 1.

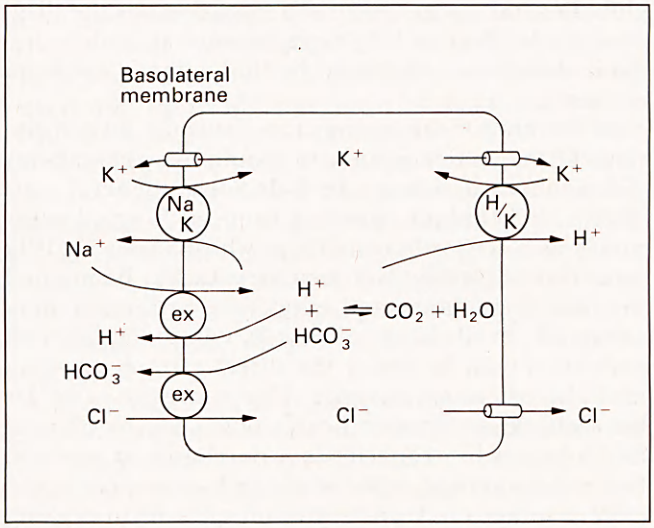
The rat proton pump has now been cloned. The enzyme has a predicted mol wt of 113kDa, with eight transmembrane spanning regions (alpha subunit). The beta subunit consists of a single transmembrane spanning region and a heavily glycosylated extracellular domain which may serve to protect from proteolytic attack the portion of the protein that protudes into the lumen of the gastric gland.

Pharmacological modulation of the parietal cell represents *the* success story of drug development of recent years. Cimetidine revolutionised the clinical management of peptic ulcer disease in the early 1970s, and created a multi-million pound market. Ranitidine has replaced cimetidine as the world's best selling drug, and famotidine is in the list of top twenty best sellers. Current interest is centred on irreversible inhibitors of the proton pump, such as omeprazole and lansoprazole, which are even more effective drugs in the treatment of acid-peptic disorders. Whether enzyme inhibitors will eventually displace receptor antagonists as the drugs of choice in the management of peptic ulcer disease and reflux oesophagitis, in the same way as, say, ACE (angiotensin converting enzyme) inhibitors have overtaken beta blockers in the therapy of hypertension, remains to be seen.

Small intestine

Professor L A Turnberg (President, Royal College of Physicians) outlined the complex regulation of enterocyte ionic secretory processes. Their control is highly complex, involving neurohumoral regulation (substance P, and VIP) as well as mast cell mediators (histamine and 5-HT), kinins and prostaglandins, all acting on specific enterocyte receptors. The Cl^- channel is one of many but is critical; secretion occurs predominantly from the crypt enterocytes, but also from the villus cells. There is a plethora of candidate intracellular messengers (cAMP, cGMP, diacylglycerol and calci-

Fig 1. *Transport model of the parietal cell.* Apical membranes contain the H^+,K^+ pump and conductive pathways for K^+ and Cl^-. Basolateral membranes contain the H^+,K^+ pump, a K^+ conductance, and separate Na^+,H^+ and Cl^-,HCO_3^- exchangers



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um), but the common denominator to all of these may be the protein kinase cascade, which eventually results in phosphorylation of critical transport proteins and activation of secretion. An intriguing issue arises from the nature of the cystic fibrosis gene; affected patients have a defect in Cl^- ion secretion, which may explain why CF patients are less severely affected by secretory diarrhoeal illnesses. This could be the basis of a survival advantage for the CF gene.

And now to the question of the day, from Dr P D **Fairclough** (St Bartholomew's Hospital, London): 'In view of the complexity of the enterocyte secretory processes, is it not surprising that a normal person produces just a single formed stool a day?!' The President was stumped!

Colon

Dr G I Sandle (Hope Hospital, Manchester) pointed out that salt and water absorption by the human colon reflects transport processes located in the apical and basolateral membranes of the surface colonocytes. In ulcerative colitis and Crohn's disease, loss or impairment of both electrogenic and electroneutral Na^+ transport mechanisms removes the driving force for water absorption. Studies with intracellular microelectrodes have shown that the cellular basis for these transport abnormalities includes (a) defective Na^+ uptake (via Na^+ channels and the Na^+Cl^- cotransporter) at the apical membrane, (b) a marked decrease in basolateral Na^+,K^+-ATPase activity, and (c) an increase in the leakiness of the colonic epithelium to monovalent ions. Diarrhoea in these patients therefore appears to reflect failure of salt and water absorption rather than stimulation of secretory processes. Nevertheless, pharmacological doses of glucocorticoid hormones (eg hydrocortisone) enhance within five hours electrogenic sodium absorption and water absorption in the inflamed distal colon of colitic patients to the same extent as in normal subjects. These changes probably follow crossover binding to and the activation of mineralocorticoid receptors in surface colonocytes. The antidiarrhoeal effects of glucocorticoids therefore reflect direct stimulation of salt and water absorption in addition to their anti-inflammatory properties.

In contrast, secretory diarrhoea is the clinical manifestation of profuse Cl^- and water secretion which originates from crypt cells of the small and large intestine, and may be triggered by a variety of cAMP- and Ca^{++}-mediated bacterial enterotoxins and neuropeptides. Recent studies using patch clamp techniques have identified voltage-sensitive low conductance channels in the basolateral membrane of human colonic crypts which are highly selective for K^+, inhibited by Ba^{++}, and activated directly by increases in intracellular cAMP and Ca^{++}. In combination with the loop diuretic-sensitive Na^+,K^+-$2Cl^-$ cotransporter and ouabain-sensitive Na^+,K^+-ATPase, these channels play a critical role in sustaining the Cl^- secretory process by hyperpolarising the cell and allowing K^+ to recycle across the basolateral membrane (Fig 2). Future studies directed at unravelling the second messenger regulation of these potassium channels may provide new pharmacological options for the treatment of a variety of secretory diarrhoeal diseases.

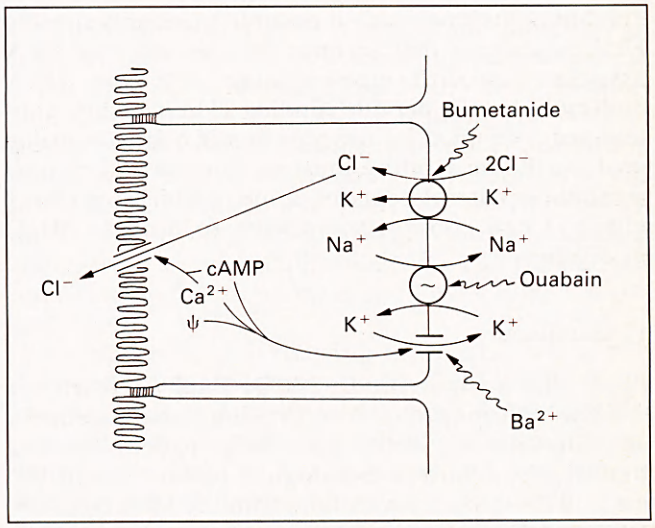


Fig 2. *Ion transport and second messengers in human colonocyte secretory function*

HLA (human leukocyte antigen) associations

Professor R I Lechler's (Royal Postgraduate Medical School, London) introduction to the molecular basis of HLA–disease associations was unusually clear for a talk on this subject. The 4 megabase pair stretch of relevant DNA is located on the short arm of chromosome 6. There are the class I (A,B,C), class III (C2,C3 etc) and class II (DR,DQ,DP) genes, the latter being the most centromeric. Many of the genes have no known function. Many are carried together, ie in linkage disequilibrium (eg A1, B8 and DR3) bewitching many premature assumptions of disease associations. Polymorphisms among HLA class II alleles are crucial to immune activation events in at least two ways: polymorphic amino acids on individual class II molecules determine whether or not specific antigenic peptides will bind and be presented on the surface of antigen-presenting cells to T lymphocytes. Furthermore, similar polymorphisms regulate the developmental selection of T cell receptor specificities during T cell differentiation and maturation in the thymus. Thus, MHC (major histocompatibility complex) class II polymorphisms control key mechanistic aspects of the immune repertoire and immune activation. Numerous population based and family studies have documented the association and linkage of certain HLA specificities with particular diseases. Recent molecular biological

developments have made it possible to identify specific HLA haplotypes that account for the observed HLA associations with autoimmune diseases. HLA molecules have a peptide binding cleft, which is continuously occupied by antigens of self origin. A major goal for the near future must be to elute and characterise these natural peptides, some of which (eg class I eluates) can control the ability to bind to MHC molecules.

Coeliac disease

From this we launched with **Dr Paul Ciclitira** (St Thomas's Hospital, London) into the immunogenetics of coeliac disease, which has a 10% incidence among first degree relatives. Serological techniques in the early 1970s showed associations with the HLA types B8, DR3 and DR7. Dr Ciclitira's recent work, aimed at determining the relationship of HLA class II alleles with coeliac disease, has shown a near 100% association of coeliac disease with HLA DQ2; the small number of patients who do not express this HLA phenotype generally express HLA DR4 (Fig 3). He suggests that DQ2 is involved in wheat gliadin antigen recognition and presentation in gluten sensitive enteropathy. The methods are elegant: the reader interested in knowing more should find Dr Ciclitira's 1991–3 papers in *Gut* and *Gastroenterology*.

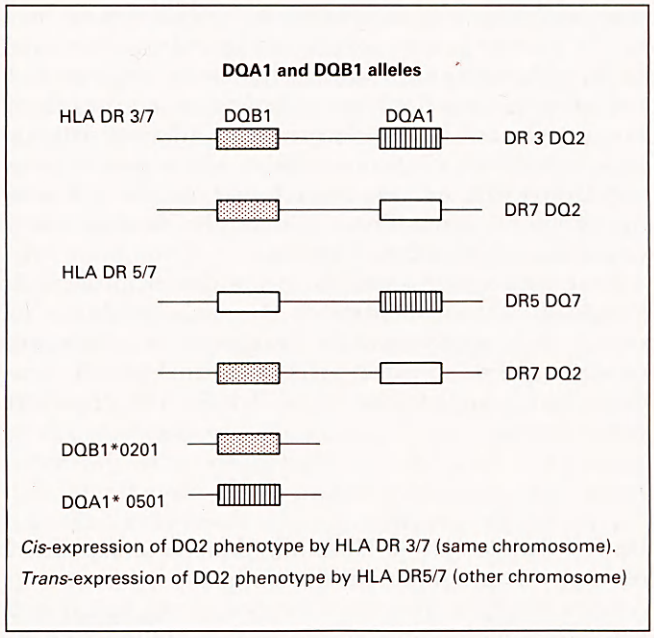


Fig 3. *Immunogenetics of coeliac disease.* Cis and trans expression of the HLA DQ2 phenotype in coeliac disease

Haemochromatosis

Professor Timothy Cox's (Addenbrooke's Hospital, Cambridge) group may be only several hundred kilobases away from the haemochromatosis gene, which he hopes is present on a YAC (yeast artificial chromosome) clone characterised by him. It is vital to spot the disease early; for example, hepatocellular carcinoma is a major risk only to those who already have a cirrhotic liver at the time of diagnosis, whereas patients treated before they are cirrhotic can look forward to a normal life expectancy. The disease is autosomal recessively inherited, a point missed by classical geneticists. In affected families the predictive value of HLA-A typing is exceedingly strong. As to the function of the elusive gene: one can still only speculate about its likely tissue expression or role. Unfortunately it is still true that 'Gold is for the mistress, silver for the maid; copper for the craftsman cunning at his trade. 'Good!' said the baron, sitting in his hall, 'But iron—cold iron—is master of them all'.' (R Kipling: 'Cold Iron').

Cancer

Tumour suppressor genes

Dr Huw Thomas (St Mary's Hospital, London) reviewed the recent giant strides in our understanding not only of familial adenomatous polyposis (FAP) but also of sporadic colorectal cancer. The underlying theme in both conditions is that loss of genetic material at specific chromosomal loci might result in tumour formation, as is the case for retinoblastoma. Following a clue from karyotype studies of a patient with FAP showing a deletion in the long arm of chromosome 5, Sir Walter Bodmer's group (1987) had demonstrated a consistent polymorphism at the specific genetic linkage site of a 5q probe. *In situ* hybridisation located the FAP gene to region 5q21-22, stimulating the search for loss of alleles on this chromosome. It was not long before the equally exciting finding of similar 5q losses in up to 40% of sporadic tumours (but not adjacent normal mucosa) was demonstrated. Vogelstein pursued this and showed that 29% of benign colonic adenomas also contained allele loss on chromosome 5; this loss of heterozygosity is purported to be an early event in colorectal carcinogenesis. Positional cloning, a method by which a gene is identified by its position rather than function, was used to identify several 5q21 genes. One of them, the MCC (mutated in colorectal cancer) gene, was shown to be somatically mutated in a subset of colorectal cancers. Another, the APC (altered in polyposis coli) gene, was shown to undergo similar somatic mutations and also to be mutated in the germline of patients with FAP (67% of unrelated cases), most of which were predicted to result in truncations of the APC protein.

These findings have major implications for the understanding of tumourigenesis: certain genes (tumour suppressor genes) are required to maintain normal or controlled cell growth. Loss of one or more

of them can lead to disordered cell growth and cancer. Automated DNA sequence analysis for the presence of mutations in these genes may well revolutionise tumour management and surveillance strategies in the future.

Tumour surveillance

Professor Nicholas Wright (Royal Postgraduate Medical School, London) asked: is there such a thing as a cancer specific epitope? The short answer is 'no'. Various candidates, including p53, MUC 1–5, growth factor receptors (eg *erb*B2) and *ras*, have failed to live up to initial expectations. However, PCR (polymerase chain reaction) of faecal DNA, using primers targeted at *ras* and APC genes, is under active study as a means of early detection of adenomas and non-invasive diagnosis of colorectal cancer.

Intestinal cancer

Cancer screening remains the thorniest of issues. **Professor J D Hardcastle** (University of Nottingham) pointed out that the haemoccult test misses 40–50% of colorectal cancers, presumably owing to the episodic nature of bleeding from some tumours. The false positive rate of 2–3% means that many unaffected individuals are needlessly investigated at great expense. One way to improve on this is to combine testing for occult blood with an endoscopic procedure, such as sigmoidoscopy; though this is not likely to be widely acceptable, it offers the likelihood of a decrease in colorectal cancer mortality by an estimated 60–70%, which lasts 10 years. Further work should concentrate on better screening tests, and high risk groups.

Gastric cancer

Professor T C Northfield (St George's Hospital, London) presented evidence in favour of screening for high risk groups: early detection may improve the prognosis. Patients who have undergone a Billroth II operation are worthy targets for serial monitoring for dysplasia around the stoma. But how often to endoscope? There is no clear answer: possibly yearly. There are no adequate data for screening for gastric cancer in pernicious anaemia, or in *H pylori* infection.

Screening programmes

Dr R Logan (University of Nottingham) discussed how screening programmes should be evaluated. Three questions need to be answered: 1. Does the screening programme actually work, ie does it reduce mortality? 2. Is the screening method acceptable to the target population? 3. If there are satisfactory answers to the foregoing questions, will the benefit be cost effective?

A randomised controlled trial is the ideal way to study the effectiveness of a screening programme. It copes with the problems of lead-time bias, duration (length-time) bias and selection bias. Case control studies are less satisfactory alternatives because of the preferential uptake of screening by health conscious individuals. Nevertheless, the most convincing evidence of the value of colorectal cancer screening comes from a recent Californian case control study of screening by rigid sigmoidoscopy (Selby *et al. N Engl J Med,* 1992).

Acceptance of screening is highest when the screened group is patients who have obvious symptoms such as colitis or dyspepsia, and lowest for the general population having faecal occult blood testing or sigmoidoscopy. Even so, greater than 50% compliance rates have usually been achieved.

In the absence of good evidence of the effectiveness of either population screening or surveillance of high risk groups, detailed economic analyses are not available. The Nottingham group (Walker *et al, J Epidemiol Community Health,* 1991) have estimated the costs incurred in their colorectal cancer screening trial and found that the costs per test and costs per cancer detected were of an order similar to that for breast cancer screening as estimated in the Forrest report. Current evidence suggests that colorectal cancer screening either by stool testing or by sigmoidoscopy can significantly reduce mortality; it is acceptable to at least half the population, and is possibly cost-effective in comparison with breast cancer screening. Screening in western countries for other gastrointestinal cancers is less effective and, with the exception of high risk groups, not worthwhile.

Ulcerative colitis

Professor J E Lennard-Jones (St Mark's Hospital, London) gave a critical assessment of surveillance for colorectal cancer in patients with ulcerative colitis (UC). One might have thought that here the situation would be a little clearer; but not so. It is accepted that the cancer risk is significant in patients with pancolitis and those who have had disease for at least eight years. Dysplasia is a qualitative measure with interobserver variation. It is patchy, giving rise to sampling errors when all that is available are endoscopic biopsies. A ridiculously large number of biopsies is required to achieve a 95% confidence level for the absence of colonic dysplasia. Dysplasia is absent in one third of patients who have developed a cancer. Fifty per cent of patients with UC with high grade dysplasia, or low grade dysplasia plus a mass lesion, already have a cancer, so these end points are too often too late. A large detailed case controlled study is needed to prove that UC screening for cancer brings any benefit. Until then, there is 'no ethical imperative to undertake cancer surveillance in our patients with colitis'.

Destructive hepatic processes

Hepatitis C

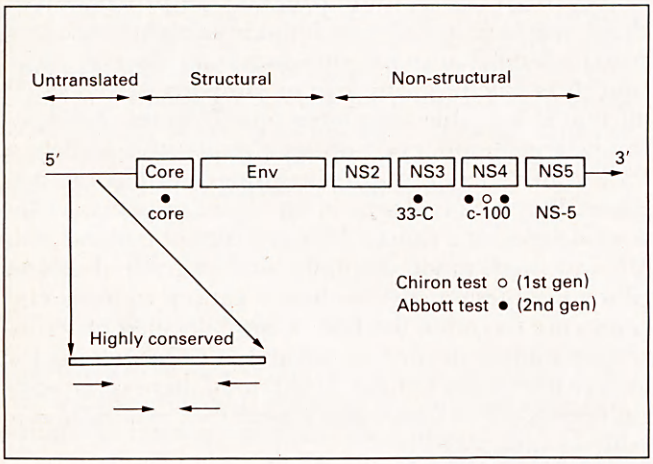
Dr G M Dusheiko (Royal Free Hospital, London) reviewed the cloning and diagnosis of hepatitis C virus (HCV). Following the development of serologic tests for hepatitis A and B in the early 1970s, it soon became clear that about 75% of post-transfusion hepatitis was due to neither of these viruses, and this was designated non-A non-B (NANB) hepatitis. In 1989 Choo *et al* cloned a viral antigen from a phage cDNA expression library derived from an infected chimpanzee's plasma using antibodies from a patient's serum as a probe, and the virus was named hepatitis C. Classical virology had failed to isolate the virus. The antigen was successfully used to develop an antibody test for hepatitis C, which was shown to account for 90% of cases of NANB hepatitis. The virus has a small RNA genome (10kb) with core, envelope and non-structural genes (Fig 4). PCR of the 5' non-coding region of the genome has been used to confirm the presence of virus RNA in infected blood; eg immunosuppressed patients have high levels of viraemia.

Groups at particularly high risk of carrying hepatitis C are haemophiliacs who received pooled factor VIII (70–100% prevalence), patients who have received multiple blood transfusions, and intravenous drug addicts (50% prevalence). The risk of sexual transmission is relatively low (but not zero). As many as 50% of cases of sporadic hepatitis C in the UK are not associated with an obvious parenteral or sexual source of infection. Worldwide there are an estimated 150 million carriers of hepatitis C.

Viraemia occurs 1–2 weeks after intravenous inoculation with infected blood. An acute hepatitis follows 5–10 weeks after inoculation, but is symptomatic in only 20% of cases. Seroconversion can only be demonstrated after 11–12 weeks, therefore a negative antibody test does not rule out acute hepatitis C. Only 30% of patients with acute hepatitis achieve a rapid biochemical resolution, and when this occurs it is followed by gradual disappearance of anti-HCV (mean time to disappearance is five years). Unfortunately 50–70% become chronic carriers and viraemia persists in approximately 95% of them despite the antibody response. Liver biopsy shows varying degrees of inflammation, including chronic persistent and chronic active hepatitis, progressing through bridging necrosis to cirrhosis; and there is a clear increase in the risk of hepatocellular carcinoma.

Interferon has been used to treat chronic hepatitis C when an active hepatitis is demonstrable either biochemically or histologically. Although it achieves normalisation of transaminases and improvement in liver histology, only 20–30% derive lasting benefit from it. **Dr Roger Williams** pointed out that not enough is known about the natural history of the disease at this stage to recommend liver biopsy in all cases; as a rough guideline, a staging biopsy is indicated if the serum AST (aspartate aminotransferase) level is greater than twice the upper limit of normal.

Fig 4. *The hepatitis C RNA genome. Single-stranded +sense RNA virus 10 kb of single open reading frame.* The highly conserved region is the target for nested PCR in the diagnosis of hepatitis C infection



Hepatitis B

Professor H C Thomas (St Mary's Hospital, London) clarified the pathogenesis of persistent and fulminant hepatitis B in the light of recent work on virus variants. In some recently studied patients with fulminant hepatitis B, HBsAg (hepatitis B surface antigen) and anti-HBe were present, rather than the HBe antigen. The messenger RNAs for both HBe and HBc antigens are coded by the same sequence in the viral genome and use the same open reading frame. A single mRNA is synthesised, but read from different start-methionines to make the e (envelope) and c (core) antigens respectively. For many years it was unclear why some patients positive for HBsAg and anti-HBe continued to be highly infective, secreting copious virus with severe chronic hepatitis and progression to cirrhosis. It was subsequently discovered that these patients have a variant of HBV in which a stop-codon mutation occurred in HBe upstream of the HBc initiation site: synthesis of e antigen failed, but that of c antigen proceeded, explaining the discordance between anti-HBe and HBeAg status. Similar stop-codon mutations in the same pre-core region have been detected in patients with fatal fulminant hepatitis B. Why some patients should develop fulminant hepatitis while others develop chronic liver disease is still a puzzle, but the variability of host immunologic factors is undoubtedly a major determinant. One possibility is that the prematurely cleaved HBe N-terminal antigen serves as a T cell recognition target; in chronic hepatitis the mutants may be initially present but are later cleared. We should all now look at HBV and probably also HCV

Table 1. Characteristics of subtypes of AI-CAH

I	Antinuclear antibody (ANA) and/or smooth muscle antibody (SMA) positive
II	Liver-kidney microsomal (LKM-1) antibody positive
	IIa: younger, anti-HCV negative IIb: older, anti-HCV positive
III	Negative for ANA, SMA and LKM-1 but positive for anti-SLA (soluble liver antigen)

AI-CAH patients who are anti-LKM positive and anti-HCV positive can be differentiated from patients with active HCV infection by PCR of serum for HCV; interferon is indicated for some of the latter but is dangerous in the former.

as viruses which evolve, even during infection within an individual, as a result of immunological pressures.

Chronic active hepatitis

Professor K-H Meyer zum Büschenfelde (University of Mainz, Germany) gave a superb account of autoimmune chronic active hepatitis and its antibody profiles. AI-CAH is a progressive inflammatory liver disease requiring early diagnosis because immunosuppressive treatment greatly improves its prognosis. The diagnostic approach has been made much easier by the establishment of a panel of marker autoantibodies that define several subgroups of the disease (Table 1).

Autoantibodies directed at other liver components are also present, in particular the asialoglycoprotein receptor (anti-ASGPR). These show specificity for all subtypes of AI-CAH (and some patients with primary biliary cirrhosis). The ASGPR may well function as a hepatic autoantigen in AI-CAH: ASGPR specific CD4+ T lymphocytes are found at the site of tissue injury, suggesting that this hepatocyte surface molecule functions as a hepatic autoantigen in AI-CAH, with a role in initiation or perpetuation of the disease. Interestingly, the anti-ASGPR titre falls as the activity of the liver disease abates with immunosuppressive therapy.

Envoi

It is a worrying prospect for the future of medical research in the UK that audience participation failed to match the level of the basic science presentations. However, the meeting livened up during the more clinical sessions; those on cancer and liver disease were particularly well received. The gap between science and practice must not be allowed to widen further.

Current opportunities in clinical research

This conference was held by the Academic Medicine Group at the Royal College of Physicians on 18 December 1992. It was designed to help doctors wondering whether to embark permanently, or even for a limited period, on a career in clinical research. The speakers, most of them already committed and successful research workers, illustrated both the satisfaction and the problems that come with such a way of life.

Like Caesar's Gaul, the conference was divided into three parts.

- Why doing clinical research is a good thing: claimed by those who have already done well.
- The difficulties in planning a research career: proclaimed by those selecting it.
- Where to find the opportunities: explained by those in whose gift they lie.

Is clinical research a good thing?

Professor David Weatherall's (Medicine, Oxford) answer was: 'yes, provided it is worthwhile work based on good and adequate training'. He pointed out that clinically orientated research covers a wide spectrum, from molecules to whole populations, from fundamental laboratory science to clinical epidemiology; but that its place has to be defended in the face of an anti-science lobby both within and without the medical profession. He wanted all undergraduate students to know enough elementary statistics to be able to assess the validity of statements in articles and textbooks. He thought that it would be ideal if doctors who wanted to do good research had at least two or three years free from regular clinical commitments to learn the necessary techniques and have time to produce worthwhile data. Those with a strong scientific bent might then go on to a full-time career as medical scientists in a research institute; the more usual academic doctors would probably do half-time research and half-time

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